Regulation of Hepatic High Density Lipoprotein Binding Protein, HB2, Expression after Administration of Simvastatin to Rabbits

Yoko Fujiwara1,2, Kazuo Kondo1, Hiroshige Itakura1, Tomoyuki Fujioka3, Yoshio Tsujita3, Hideaki Kurata4, Noel Fidge5, and Akiyo Matsumoto1

1The National Institute of Health and Nutrition, Tokyo, Japan.
2Division of Nutrition and Food Science, Ochanomizu University, Tokyo, Japan.
3Pharmacology and Molecular Biology Research Laboratories, Sankyo Co. Ltd., Tokyo, Japan.
4Jikei University, School of Medicine, Tokyo, Japan.
5Baker Medical Research Institute, Melbourne, Australia.

HB2, a candidate HDL receptor, is quite distinct from other HDL receptors in its structure. However, while changes in cellular cholesterol content, or a reduction in cholesterol biosynthesis accompany corresponding changes in HB2 expression, the level at which these changes occur have not been determined and the regulation and the function of HB2 remain uncertain. In order to further investigate the regulation of HB2, we administered simvastatin to rabbits to reduce cholesterol biosynthesis and follow changes in HB2 mRNA in various tissues. Six rabbits were given 15 mg/kg of simvastatin by oral administration daily and another six rabbits were given the same volume of saline as a control, for 21 days. They were then sacrificed to obtain samples of blood, liver, lung, jejunum and brain. Simvastatin reduced plasma total cholesterol by 47% and free cholesterol concentrations in liver and lung by 25 and 10%, respectively. Northern blot analysis showed that simvastatin lowered the expression of HB2 significantly in the liver and lung by 54% and 42% respectively but not in the jejunum or brain. These results support the findings of a previous study showing that HDL binding activity of both HB1 and HB2, which was determined by ligand blotting using HDL3 as a ligand, were reduced after administering cholesterol lowering agents. (Arteriosclerosis, 10 : 1045-1050, 1990). The present study suggests that simvastatin down-regulated HB2 at the transcriptional stage. Although the complete physiological function of HB2 is unclear, it appears to play some role in the cholesterol metabolism, warranting further studies to elucidate the nature of this interaction. J Atheroscler Thromb, 2000 ; 7 : 203-208.

Key words : HDL, Receptor, Simvastatin, Rabbit

Introduction

The incidence of premature artery wall disease is lowered in the presence of high levels of circulating high density lipoprotein (HDL) suggesting that HDL may protect individuals against atherosclerosis (1). Part of this protection may be attributed to the participation of HDL in the reverse cholesterol transport pathway, a mechanism that reduces the accumulation of cholesterol in the arterial wall and the narrowing which subsequently occurs (2). We have purified two proteins, HB1 and HB2 (HDL binding proteins 1 and 2), from rat liver (3) and succeeded in...
cloning HB2 and have reported its cDNA sequence and characterized some of its properties (4). As a candidate HDL receptor, it is quite distinct from SR-BI or other HDL binding proteins previously reported (5, 6), and shows significantly homology with the adhesion molecules ALCAM and BEN of the immunoglobulin superfamily (7, 8). The structure of HB2 comprises a 32-amino acid cytoplasmic domain, 24-amino acid hydrophobic transmembrane domain and approximately 500 residues of an extracellular domain terminating in the NH2 residues. When HB2 cDNA was transfected into HepG2 cells and Chinese hamster ovary cells (CHO cells), specific HDL3 binding activity increased by 80-100%. THP-1 cells treated with phorbol 12-myristate 13-acetate (PMA) resulted in a striking increase in HB2 mRNA, an expression that was subsequently reduced by cholesterol loading the cells with acetyl LDL (4). These results suggested that, distinct from the properties of a cell adhesion molecule, HB2 may have an alternative role that is related to the cell lipid metabolism or processing of HDL, but the details of this lipid associated function of HB2 is not known as yet.

In order to further pursue a possibility (9) that cell cholesterol synthesis may be related to the expression of HB2, we have treated rabbits with simvastatin, an HMG CoA reductase inhibitor, and determined the effect of lowering cholesterol synthesis on HB2 expression.

Materials and Methods

Simvastatin was used as the lactone form transformed to the sodium salt of the hydroxy-acid (active form). All other chemicals used were of analytical grade.

Animals and diet

Twelve male Japanese white rabbits (average weight 3 kg) were purchased from Oriental Yeast Co. Ltd. (Tokyo, Japan) and fed a commercial chow, RC-4 pellet (Oriental Yeast Co Ltd.) throughout the experiments. Six rabbits were given 15 mg/kg of simvastatin by oral administration from each rabbit by cardiac puncture under anesthesia by pentobarbital sodium (Abbott Laboratories, USA) after overnight fasting. Liver, lung, jejunum and brain were harvested for RNA extraction. All the tissues were immediately transferred into liquid nitrogen for storage before the extraction of RNA as below.

Measurement of HB2 mRNA expression

Frozen tissue (200 mg) was broken into small pieces and homogenized in 5 M of guanidium thiocyanate solution using a polytron homogenizer. Total RNA was prepared as described elsewhere (10). Fifteen micrograms of total RNA was isolated by 1% agarose gel electrophoresis including formamide, transferred to a nylon membrane (0.45 μm Nytran Schleicher & Scheull, Sassel W.Germany) and fixed using a UV cross linker (Stratagene, Cambridge). After 2-hour prehybridization, the membrane was hybridized overnight at 42°C in the presence of a probe, rat HB2 cDNA fragment (423-1,032 nt, 610 bp) labeled with [α-32P]dCTP (NEN, USA) using a random priming labeling kit (Takara Biomedicals, Kyoto, Japan). The membrane was washed 3 times with 2 x SSC (containing 0.1% SDS) at 42°C, twice by the same buffer for 30 minutes at 50°C and one final wash with 0.1 x SSC at 50°C for 30 minutes. HB2 mRNA was determined by autoradiography and analyzed by image analysis (BAS 2000, Fuji Film Co., Japan). The same process was repeated using a fragment of glyceraldehydes phosphate dehydrogenase (GAPDH) as a control. Levels of HB2 mRNA were quantitated by estimation of the photosensitized luminescence per area (PSL/A) of the corresponding band by BAS 2000. The data obtained were normalized for GAPDH level.

Determination of lipid concentration in plasma and tissues

Plasma was obtained by centrifugation of rabbit blood. Concentrations of total cholesterol, free cholesterol, phospholipids, triglyceride and HDL-cholesterol in plasma were determined enzymatically using an autoanalyzer (HITACHI 7650). HDL-cholesterol was determined using the MgCl2-phosphotungstic acid precipitation method. VLDL+LDL-cholesterol level was estimated by the difference between total cholesterol and HDL-cholesterol. Lipids of liver and lung were extracted by the method of Folch et al. (11). The concentration of free cholesterol was measured by HPLC equipped with a HITACHI L6300 Intelligent Pump (HITACHI, Japan) and an L4200H UV-VIS Detector P2500 Chromato Integrator (HITACHI, Japan). HPLC was performed using μBONDASPHERE (5 μC18, 3.9 x 150 mm, Waters, Japan) with acetonitrile/2-propanol (1 : 1 vol/vol) as the mobile phase.

Statistics

The Student’s t test was used to compare the simvastatin treated samples with the controls.

Results

Table 1 shows the changes in serum lipid concentrations after administration of simvastatin. The statin had no effect on the weights of rabbits during the test period, but plasma total cholesterol and free cholesterol was significantly reduced by 39%. VLDL and LDL-cholesterol were significantly decreased by 47%. Although HDL-cholesterol tended to be reduced, the ratio of HDL-cholesterol: total cholesterol was slightly higher in simvastatin treated rabbits than in control (61.3% and 55.3%, respectively).

Fig. 1 shows the changes in cholesterol concentrations...
Regulation of HB2 Expression with Simvastatin

Table 1. Lipid concentration in serum of rabbits treated with simvastatin.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Simvastatin</th>
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<tbody>
<tr>
<td></td>
<td>mg/dl</td>
<td>mg/dl</td>
</tr>
<tr>
<td>T-Chol</td>
<td>34.9±4.9</td>
<td>21.4±1.6*</td>
</tr>
<tr>
<td>F-Chol</td>
<td>7.2±1.3</td>
<td>4.4±0.4**</td>
</tr>
<tr>
<td>TG</td>
<td>28.5±4.8</td>
<td>30.9±6.1</td>
</tr>
<tr>
<td>PL</td>
<td>66.0±7.9</td>
<td>56.3±3.8</td>
</tr>
<tr>
<td>CE</td>
<td>27.7±3.6</td>
<td>17.0±1.2*</td>
</tr>
<tr>
<td>HDL-Chol</td>
<td>19.3±3.5</td>
<td>13.1±1.4</td>
</tr>
<tr>
<td>VLDL+LDL-Chol</td>
<td>15.6±2.6</td>
<td>8.1±0.8*</td>
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Significant differences from control: *; p<0.05, **; p<0.01 (n=6).

Fig. 1. Effect of simvastatin on free cholesterol concentrations in liver and lung of rabbits. Rabbits were treated with 15 mg/kg of simvastatin or saline (Control) for 21 days. Total lipids were extracted from liver and lung of rabbits by the Folch method (see text). Free cholesterol concentrations were measured by HPLC as described in Materials and Methods. Values are mean±SD *; significant difference from Control, p<0.05 (n=6).

Fig. 2. Northern blot hybridization of HB2 mRNA in rabbit with rat HB2 cDNA probe. 15 μg of total RNA extracted from liver (Liv), lung (Lun), jejunum (Jej) and brain (Bra) of rabbit were separated by 1% agarose gel electrophoresis. After transferring to a nylon membrane, RNA blot was hybridized using rat HB2 cDNA probe. (A) Autoradiography after 48 hr exposure (B) RNAs stained with EtBr.

Table 1: Lipid concentration in serum of rabbits treated with simvastatin.

- T-Chol: 34.9±4.9 mg/dl, 21.4±1.6* mg/dl
- F-Chol: 7.2±1.3 mg/dl, 4.4±0.4** mg/dl
- TG: 28.5±4.8 mg/dl, 30.9±6.1 mg/dl
- PL: 66.0±7.9 mg/dl, 56.3±3.8 mg/dl
- CE: 27.7±3.6 mg/dl, 17.0±1.2* mg/dl
- HDL-Chol: 19.3±3.5 mg/dl, 13.1±1.4 mg/dl
- VLDL+LDL-Chol: 15.6±2.6 mg/dl, 8.1±0.8* mg/dl

Significant differences from control: *; p<0.05, **; p<0.01 (n=6).

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A Northern blot analysis of HB2 mRNA in liver of rabbits treated with simvastatin is shown in Fig. 3. Figs. 4 and 5 show the levels of HB2 mRNA normalized by GAPDH. Simvastatin treatment significantly decreased the expression of HB2 in the liver by 54%. As shown in Fig. 4B, simvastatin also significantly lowered the HB2 expression in lung by 42%, but did not produce changes in the jejunum and brain (Fig. 5A and B).
Discussion

HB2 is one of two candidate HDL receptors purified from rat and human liver (3) and has been cloned and sequenced (4). Mathai et al. (9) reported previously that the binding activities of HB1 and HB2 were reduced significantly in the livers of rats fed simvastatin alone or simvastatin with cholestyramine, using ligand blot analysis. Having postulated that the decrease in binding activity was most likely due to a decrease in expression of HB2 protein that followed a reduction in cholesterol synthesis or as a direct result of simvastatin, we sought clarification of the regulatory process by measuring HB2 mRNA levels in various rabbit tissues after treating the animals with simvastatin.

The results of the present study essentially confirm the previous finding (9) but in addition show that the reduction in HB2 follows a corresponding down-regulation of HB2 mRNA in response to simvastatin. This response was most evident in the liver and lung, with little change seen in either the brain or jejunum. The data suggest that in the lung and liver, biochemical events following statin treatment lead to transcriptional regulation of HB2. The changes in HB2 expression accompanied a lowering of HDL cholesterol by approx 33% and VLDL + LDL cholesterol by approx 50%. Whether or not these changes in plasma lipids influenced the regulation of HB2 (and HB1) is unknown but an alternative suggestion, that simvastatin acting through cellular pathways influences transcription of HB2 mRNA and subsequently, HB2 levels, appears more likely. A positive correlation between HDL cholesterol and HB2 mRNA levels suggests that HB2 is one of many factors that regulate plasma HDL-cholesterol levels. Although the function of HB2 is not known, HB2 may regulate cellular cholesterol by inhibiting cholesterol efflux from the liver when cholesterol synthesis is down-regulated.

HMG CoA reductase inhibitors are known to increase HDL cholesterol levels in humans (12-14), while they appear to decrease not only total cholesterol but also HDL-cholesterol in most experimental animals (15-17). In this study, rabbits were fed a normal diet without additional cholesterol during the experimental period. The ratio of HDL-cholesterol to total cholesterol was higher in the simvastatin treated group, although HDL cholesterol per se tended to be decreased by simvastatin. Meijer et al. (18) reported that simvastatin lowered serum cholesterol ester transfer protein (CEPT) but not lecithin : cholesterol acyltransferase (LCAT) in rabbit, so it is possible that the rise in proportion of plasma HDL shown here may have been the result of lowered CETP activity, although a reduction in HDL binding to HB2 in tissues affected by simvastatin cannot be excluded and may have contributed to the rise.

We have reported that THP-1 cells treated with PMA resulted in a striking increase in HB2 mRNA and that this rise was reduced by cholesterol loading the cells with acetyl LDL (4). Together with this data, the present studies suggest that HB2 levels may be related to changes in the cholesterol metabolism. These functions are distinct from those related to uptake of cholesterol ester (19) and cholesterol efflux that are mediated by SR-BI (20).

The evidence implicating HB2 (and HB1) in HDL physiology arose from observations that both these membrane proteins bind HDL and apoAI or apoAII, but not LDL, in ligand blot studies (3). In a more recent study, it was also shown that human monocye specifically bind HDL and apoAI, and that there is a strong positive correlation between monocyte HB2 and circulating HDL (21). Additionally, we have recently identified a domain on the extracellular region of HB2 that specifically binds HDL or apoAI, but not LDL (unpublished observations), strengthening evidence for a role of HB2 in some cellular pathway that is influenced by interaction with HDL or its apoprotein moieties. The present study has shown that, either through a direct effect on transcription or via changes in sterol biosynthesis following statin treatment, HB2 expression is down regulated, encouraging further investigation of its role in lipid metabolism.

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References

(7) Bowen MA, Patel DD, Li X, Morel R, Malacko AR, Wang W, Marquardt H, Neubauer M, Pesando JM, Francke U, Havnes BF, and Aruffo A: Cloning, mapping, and char-

(8) Pouquie O, Corbel C, Le Caer JP, Rossier J, and LeDouarin NM: BEN, a surface glycoprotein of the immunoglobulin superfamily, is expressed in a variety of developing systems. Proc Natl Acad Sci USA, 89: 5261-5265, 1992

(9) Mathai D, Fidge N, Tozuka M, and Mitchell A: Regulation of hepatic high density lipoprotein binding proteins after administration of simvastatin and cholestyramine to rats. Arteriosclerosis, 10: 1045-1050, 1990


(13) The Pravastatin Multinational Study Group For Cardiac Risk Patients: Effects of pravastatin in patients with serum total cholesterol levels from 5.2 to 7.6 mmol/liter (200 to 300 mg/dl) plus two additional atherosclerotic risk factors. Am J Cardiol, 72: 1031-1037, 1993


